Protocol for adhesion to plastic surface of *Listeria monocytogenes* grown under normal and NaCl stress conditions

Procedure for making chamber *(this is already prepared for you to use during the exercise)*

1. Prepare materials for making top part of the chamber (Fig. 1)

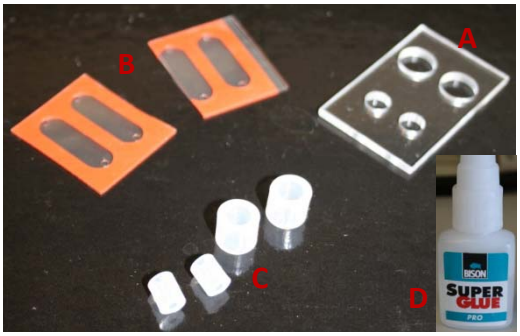

Figure 1. Materials for preparation of chambers. A: Premade Plexiglas, B: CoverWell Perfusion chamber cut in half, C: inlet and outlet tubes and D: Super glue

2. Cut the 4-chamber CoverWell perfusion chamber into two pieces (Fig. 2A). Take the Plexiglas (Fig. 2B) and glue it to CoverWell perfusion chamber making sure that the holes are in the center (Fig. 2C).
3. Glue inlet (small tubes) and outlet (bigger tubes) tubes to the Plexiglas (Fig. 2D).
4. Let them dry overnight (Fig. 2E).

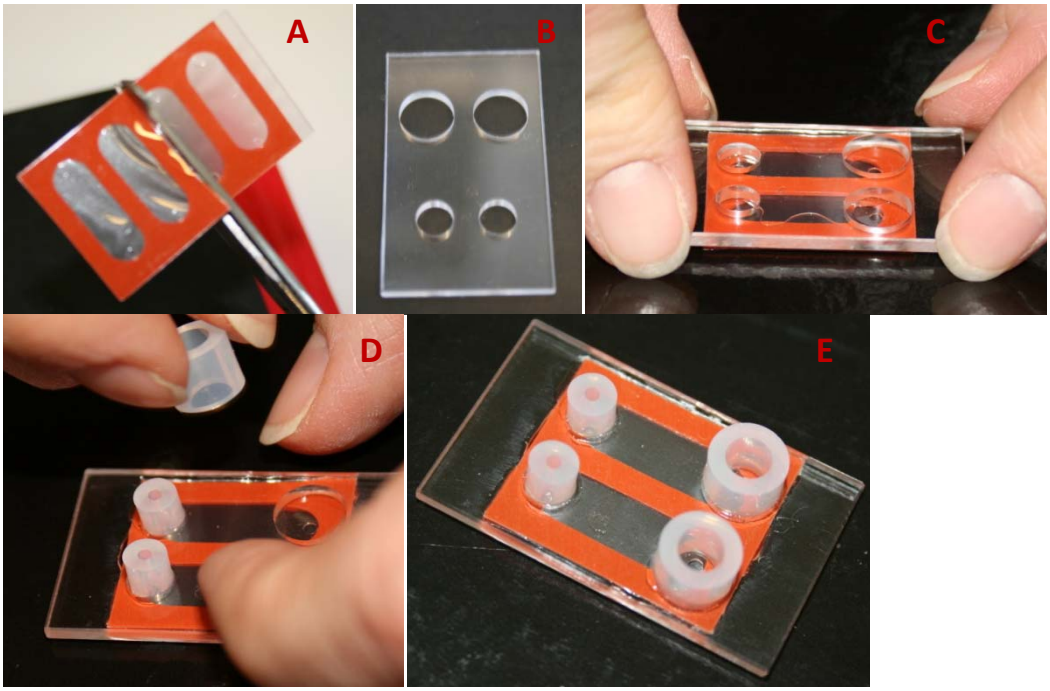

Figure 2. Construction of the top part of CoverWell perfusion chamber.

Preparation of cultures:

(the incubation of the cultures will be prepared for you in advance. The exercise starts at step 5)

Preparation of strains grown under normal condition (group 1 strain: EGDE, group 2 strain: LM 412)

1. Take 25µl from a batch freeze culture (stored at -80°C) and inoculate in 10 ml BHI (Brain Heart Infusion) medium (18.5g BHI in 500mL of distilled water, autoclaved at 121˚C for 15 minutes).
2. Inoculate overnight (around 18 hours) at 37°C with agitation
3. Take 25 µl of the culture and re-inoculate in 10 ml BHI.
4. Incubate at 37°C with agitation for 22 ± 2 hours.
5. Check the OD of cells. In a cuvette add 900uL of buffer (0.1M citric acid/0.2M Na_2HPO_4 buffer pH 6.6) and 100uL of cells. Place the cuvette in spectrophotometer and measure absorbance at 600nm
6. Dilute the culture to OD_{600} = 0.100 in buffer to a final volume of 40 ml.
7. Put the contents in 50 ml falcon tubes. This solution will be used in running the flow experiment

$$\frac{0.100\ x\ 40\ ml}{measured\ OD_{600}} = ml\ of\ ON\ culture\ needed\ for\ 40\ ml\ total\ volume$$

Preparation of strains grown under NaCl stress condition (group 1 strain: EGDE, group 2 strain: LM 412)

1. Take 25µl from a batch freeze culture (stored at -80°C) and inoculate in 10 ml BHI (Brain Heart Infusion) medium (37g BHI in 1L of distilled water, autoclaved at 121˚C for 15 minutes).
2. Inoculate overnight (around 18 hours) at 37°C with agitation
3. Take 25 µl of the culture and re-inoculate in 10 ml BHI with 5%NaCl (18.5g BHI, 25g NaCl and 500mL distilled water).
4. Incubate at 37°C with agitation for 22 ± 2 hours.
5. Check the OD of cells. In a cuvette add 900uL of buffer (0.1M citric acid/0.2M Na_2HPO_4 buffer pH 6.6) and 100uL of cells. Place the cuvette in spectrophotometer and measure absorbance at 600nm
6. Dilute the culture to OD_{600} = 0.100 in buffer to a final volume of 40 ml.
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Procedure for adhesion assay

1. PVC slides are placed in a solution of 70% EtOH + 1% 0.1M HCl overnight
2. Take a slide from the solution containing 70% ETOH + 1% 0.1M HCl
3. Rinse it carefully with distilled water and let it dry
4. Assemble the chamber by adding the "Suber Lube" on the rubber of the chamber to avoid leakage and stick the PVC slide. Place the chamber in the metal microscope holder (Fig. 3).

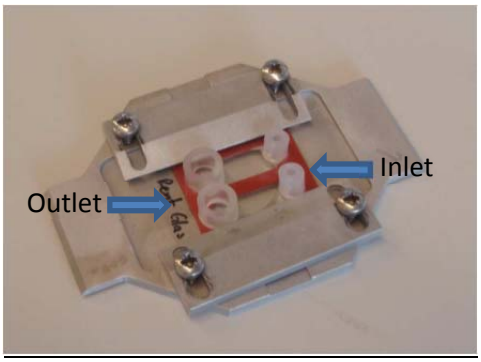

Figure 3

5. Set-up the pumps. Set the inlet pump at 0 (low flow, 0.0505 pa). The outlet pump should be set at around 600 (high flow, 0.7260 pa), higher flow than the pump used at the inlet of the chamber.
6. Start the flow by running the solution to be tested in the whole set-up to avoid having bubbles during the experiment.
7. Attach the inlet and outlet tubes in the chamber (Fig. 3) and start to run the flow. Start the timer and take the first set of pictures. Pictures are taken at 3 locations same field every time with the use of the program for the microscope stage. Don't forget to save the pictures.
8. Take pictures at 0, 5, 10, 15 minutes.
9. Clean the pump system by running 70% ethanol for 5 minutes, followed by distilled water for 5 minutes.
10. The cells which adhered are counted manually by looking at the obtained pictures.
11. Compute the initial adhesion rate.

    Formula:

    Average of the counted number of cells x 26082 = adhering cells/cm^2

    Initial adhesion rate is the slope of adhering cells/cm^2 over time: $IAR = \frac{\Delta y}{\Delta x}$

    Note:

    The value 26082 is the computed area of the chamber